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Interlaboratory Comparison of PCR-Based Identification and Genogrouping of *Neisseria meningitidis*

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Twenty clinical samples (18 cerebrospinal fluid samples and 2 articular fluid samples) were sent to 11 meningococcus reference centers located in 11 different countries. Ten of these laboratories are participating in the EU-MenNet program (a European Union-funded program) and are members of the European Monitoring Group on Meningococci. The remaining laboratory was located in Burkina Faso. *Neisseria meningitidis* was sought by detecting several meningococcus-specific genes (*crgA*, *ctrA*, 16S rRNA, and *porA*). The PCR-based nonculture method for the detection of *N. meningitidis* gave similar results between participants with a mean sensitivity and specificity of 89.7 and 92.7%, respectively. Most of the laboratories also performed genogrouping assays (*siaD* and *mynB*/*sacC*). The performance of genogrouping was more variable between laboratories, with a mean sensitivity of 72.7%. Genogroup B gave the best correlation between participants, as all laboratories routinely perform this PCR. The results for genogroups A and W135 were less similar between the eight participating laboratories that performed these PCRs.

In cases of suspected invasive infection due to *Neisseria meningitidis*, measures must be taken promptly to confirm the diagnosis, to administer appropriate antimicrobial chemotherapy, and to prevent secondary cases among close contacts (by vaccination and/or chemoprophylaxis). This requires the rapid identification and characterization of strains of *N. meningitidis*. Strains from serogroups A, B, C, Y, and W135 are responsible for over 99% of invasive meningococcal infections in Europe (26). It is essential to determine the serogroup involved (capsular immunoreactivity) before implementing vaccination strategies, as the currently available vaccines are based on capsular polysaccharides and vaccines are only available against strains of serogroups A, C, Y, and W135. Culture-based confirmation methods are hindered by the inability to isolate bacteria following early antibiotic treatment, which is recommended whenever meningococcal infection is suspected (7). Conventional non-culture-based methods, such as the latex agglutination method, can be used for diagnosis and serogrouping. Recent studies demonstrated that the sensitivity of the latex agglutination method can be improved by ultrasound treatment. However, a specific ultrasonic instrument is necessary, and some experience is required in interpreting results (15, 28, 31).

PCR-based methods for the diagnosis of meningococcal infections have recently been developed. The PCR approach has proven to be useful for meningitis surveillance even in remote areas of the African meningitis belt, such as Niger and Sudan, where culture-based identification methods cannot usually be implemented (18, 29). The most frequently tested specimens are blood and cerebrospinal fluid (CSF). However, other samples can also be tested depending on the clinical presentation, such as purpuric lesion biopsies and synovial and pericardial fluids. Several different strategies are currently used and usually involve a two-step process: (i) detection of meningococcal DNA and (ii) genogrouping to identify serogroups. The detection of *N. meningitidis* DNA may involve the amplification of specific or universal sequences within conserved genes such as the 16S rRNA gene and sequencing of the PCR product to identify the bacterial species (2, 10, 20, 25). PCR strategies that target *N. meningitidis*-specific genes are also used to detect meningococcal DNA. Several

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TABLE 1. Samples and results from all participating laboratories[a]

EU-MENET no.	Yr	Age/sex	Site	Culture/latex/smear result	Result from laboratory no.[e]:											Identification consensus (%)	Genogrouping consensus (%)
					L1[b]	L2[b]	L3	L4	L5[c]	L6	L7	L8	L9	L10[b]	L11		
01	2000	NA/NA	CSF	Neg	NmB	NmC	NmB	NmB	NmB	NmB	NmB	NmB	NmB	NmB	NmB	100 (Nm)	91 (NmB)
02	2000	NA/NA	CSF	Neg	NmB	NmB	NmB	NmB	NmB	NmB	NmB	NmB	NmB	NmB	NmB	100 (Nm)	100 (NmB)
03	2000	NA/NA	CSF	Neg	NmB	Nm	NmB	NmB	NmB	NmB	NmB	NmB	NmB	NmB	NmB	100 (Nm)	100 (NmB)
04	2000	10 mo/M	CSF	Sp (latex)	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	91 (Neg)	91 (Neg)
05	2000	46 yr/F	CSF	NmB (culture)	NmB	NmB	NmB	NmB	NmB	NmB	NmB	NmB	Nm	NmB	Neg	91 (Nm)	82 (NmB)
06	2000	30 yr/F	SynF	NmW (culture)	Nm	Nm	NmW	Neg	NmW	Neg	NmW	NmB[d]	Neg	Nm	Neg	64 (Nm)	27 (NmW)
07	2000	2 yr/F	CSF	NmB (culture)	NmB	NmB	NmB	NmB	NmB	NmB	NmB	NmB	NmB	NmB	Neg	91 (Nm)	91 (NmB)
08	2000	4 mo/M	CSF	Gram-negative cocci	NmB	NmB	NmB	NmB	NmB	NmB	NmB	NmB	Nm	NmB	NmB	100 (Nm)	91 (NmB)
09	2000	10 yr/M	CSF	NmC (culture)	NmC	Nm	NmC	NmC	NmC	NmC	Nm	Nm	Neg	NmC	Neg	82 (Nm)	55 (NmC)
10	2001	19 yr/M	CSF	NmY (culture)	Nm	Nm	NmY	NmY	NmY	NmY	NmY	Nm	Nm	Nm	Neg	91 (Nm)	45 (NmY)
11	2001	8 yr/M	CSF	Neg	Nm	NmC	NmW	NmW	NmW	NmW	NmW	NmW	NmW	Nm	NmW	100 (Nm)	73 (NmW)
12	2001	NA/M	CSF	Gram-positive cocci	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	91 (Neg)	91 (Neg)
13	2001	13 yr/M	CSF	NmA (latex)	Nm	Nm	NmA	NmA	NmW	NmA[d]	Neg	NmA	NmA	Nm	Nm	91 (Nm)	45 (NmA)
14	2001	4 yr/M	CSF	Neg	Nm	Nm	NmA	NmA	NmW	NmA[d]	Neg	NmA[d]	NmA	Nm	Neg	82 (Nm)	45 (NmA)
15	2001	5 mo/M	CSF	Neg	Nm	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	ND	Neg	82 (Neg)	82 (Neg)
16	2002	19 yr/F	SynF	Neg	NmC	Nm	NmC	NmC	NmC	NmC	NmC	NmC	NmC	NmC	Nm	100 (Nm)	82 (NmC)
17	2002	22 mo/M	CSF	Neg	NmB	Nm	NmB	NmB	NmB	NmB	Nm	Neg	Neg	NmB	Neg	73 (Nm)	55 (NmB)
18	2002	15 yr/M	CSF	Neg	NmB	Nm	NmB	NmB	NmB	NmB	NmB	NmB	Neg	NmB	Neg	82 (Nm)	73 (NmB)
19	2002	20 yr/M	CSF	NmC (culture)	Neg	Nm	NmC	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	82 (Neg)	82 (Neg)
20	2003	3.5 yr/F	CSF	Neg	Neg	Nm	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	91 (Neg)	91 (Neg)

[a] SymF, synovial fluids; ND, not determined; Neg, no amplification, negative; Nm, *N. meningitidis*; NmA, *N. meningitidis* serogroup/genogroup A; NmB, *N. meningitidis* serogroup/genogroup B; NmC, *N. meningitidis* serogroup/genogroup C; NmW135, *N. meningitidis* serogroup/genogroup W135; NmY, *N. meningitidis* serogroup/genogroup Y; NA, not available. Sp, *S. pneumoniae*; M, male; F, female.

[b] These laboratories only performed genogrouping for seorgroups B and C.

[c] This laboratory performed the analysis independently twice and obtained identical results.

[d] Genogroup was suggested on the basis of *porA* sequencing.

[e] Percentages of identification consensus for each laboratory are as follows: L1, 95%; L2, 90%; L3, 95%; L4, 95%; L5, 100%; L6, 95%; L7, 90%; L8, 95%; L9, 80%; L10, 90%; L11, 55%. Percentages of genogrouping consensus for each laboratory are as follows: L1, 60%; L2, 30%; L3, 95%; L4, 95%; L5, 90%; L6, 95%; L7, 80%; L8, 80%; L9, 65%; L10, 65%; L11, 45%.

chromosomal loci have been targeted, including the multicopy insertion sequence IS*1106* (17), the gene encoding dihydropteroate synthase (*dhps*) (21), the major porin genes *porA* and *porB* (8, 25, 35), the *ctrA* gene, which encodes an outer membrane protein involved in capsule transport (9, 17, 19, 30), and the *crgA* gene, which encodes a transcriptional regulator belonging to the LysR family (33). When meningococcal DNA is detected, the serogroup can be determined by genogrouping. Several PCR-based genogrouping assays have been developed for strains belonging to serogroups A, B, C, Y, W135, X, Z, and 29E (3–5, 12, 25, 33). The *siaD* gene encodes the polysialyltransferase responsible for the polymerization of homopolymers or heteropolymers of sialic acid-containing polysaccharides in strains belonging to serogroups B, C, Y, and W135 (14). Serogroup-specific alleles of the *siaD* gene can be detected by PCR with oligonucleotides specific for each serogroup. Serogroup A strains can be identified by amplifying the *mynB*/*sacC* gene (25, 27, 33), which is probably responsible for the polymerization of *N*-acetyl-D-mannosamine phosphate (32). No nonculture standard methods are yet available for the detection and typing of *N. meningitidis*. To implement such universal tools, we first analyzed the performance of the currently available PCR-based assays. For this purpose, we compared the approaches used in 10 laboratories participating in the EU-MenNet program (a European Union-funded program) and belonging to the European Monitoring Group on Meningococci and in the laboratory of the Centre Muraz at Bobo Dioulasso in Burkina Faso.

MATERIALS AND METHODS

Samples and conventional bacteriology. Twenty biological samples (18 CSF and 2 synovial fluid samples) were obtained from 20 different patients who were admitted to several hospitals with clinically diagnosed meningitis or arthritis. Samples were named MenNet01 to MenNet20 (Table 1). All patients had two or more of the following clinical symptoms or signs: fever, meningismus, purpura, and arthralgia. The French Reference Laboratory for Meningococci analyzed all samples by conventional bacteriology techniques (smear examination, culture, and antigen detection). The Pastorex (Bio-Rad, Marnes-La-Coquette, France) latex agglutination kit was used to detect capsular antigens and to determine the serogroup of each strain. This kit contains specific antibody-coated latex beads for the detection of *N. meningitidis* serogroups A, B, C, both Y/W135, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. Samples were cultured, and bacteria were isolated by using standard methods (16).

Sample preparation for molecular diagnosis. Samples were subjected to one freeze-thaw cycle, heated at 100°C for 3 min, and then centrifuged for 5 min at 10,000 × *g*. Two hundred microliters of each supernatant was shipped at room temperature to the participating laboratories in 11 different countries: Austria, Burkina Faso, Czech Republic, England, France, Germany, Greece, Ireland, Norway, Scotland, and Sweden. Each laboratory performed its in-house PCR assays that targeted a range of meningococcal genes: (16S rRNA, *crgA*, *ctrA*, *porA*, and IS*1106*) (4–6, 10, 12, 25, 33, 34) (Table 2). Laboratories were named L1 through L11 (Table 1). Genogrouping was performed with oligonucleotides specific for the *siaD* and *mynB*/*sacC* genes (Table 3). In two laboratories, the serogroup of genogrouping-negative samples was inferred on the basis of the sequence of the VR1 and VR2 regions of the *porA* gene. Particular sequence combinations within these two regions are associated with certain serogroups, e.g., VR1–VR20 and VR2–VR9 are associated with serogroup A strains (24).

Data analysis. The results were analyzed by using Microsoft Excel. The modal PCR result (consensus) was determined for each sample. The performance of the PCR-based methods was calculated by comparing the modal PCR results to the results obtained with conventional bacteriology techniques. The performance of the different gene targets was calculated by using the modal results as the reference. The results from the participating laboratories were compared by using the modal results as the reference. The sensitivity and specificity of *N.*

TABLE 2. Oligonucleotides used for identification of *N. meningitidis*

Laboratory(ies)	Target gene for identification	Oligonucleotide sequences (5′–3′)	Size (bp)
		GCTGCGGTAGGTGGTTCAA	
L1, L2, L5	*ctrA*	TTGTCGCGGATTTGCAACTA	111
		CATTGCCACGTGTCAGCTGCACAT (probe)	
L3, L7, L9,[c] L11	*crgA*	GCTGGCGCCGCTGGCAACAAAATTC	230
		CTTCTGCAGATTGCGGCGTGCCGT	
	IS*1106*[b]	ATTATTCAGACCGCCGGCAG	650
		CCGATAATCAGGCATCCG	
	16S rRNA[c]	AACT(AC)CGTGCCAGCAGCCGCGGTAA (outer)	
		AAGGAGGTGATCCA(AG)CCGCA(GC)(GC)TTC (outer)	1,031
		TGTTGGGCAACCTGATTG (seminested)	
		AAGGAGGTGATCCA(AG)CCGCA(GC)(GC)TTC (seminested)	710
L4	16S rRNA	TGTTGGGCAACCTGATTG	711
		TGATCCA(G/A)CCGCA(G/C)(G/C)TTC	
L6	16S rRNA	AGTTTGATCHTGGCTCAG[a]	796
		GGACTACHAGGGTATCTAAT	
L8[c]	*porA*	AAACTTACCGCCCTCGTA (outer)	Variable
		TTAGAATTTGTGGCGCAAACCGAC (outer)	
		CCGCACTGCCGCTTGCGG (nested)	Variable
		CGCATATTTAAAGGCATA (nested)	
L10	*ctrA*	TTGTGTGGAAGTTTAATTGTAGGATGC	89
		TCAGATTGTTGCCCTAAAGAGACA	
		TCCTTCATCAGGCCCCAGCG (probe)	

[a] h = A, C, or T not G.
[b] This laboratory additionally used IS*1106* for identification.
[c] These two laboratories additionally used the same 16S rRNA gene oligonucleotides for identification.

meningitidis identification were calculated by using a two-way table as the percentage of samples that gave positive and negative consensus results in each laboratory, respectively.

RESULTS

Conventional bacteriological analysis. Samples were collected from patients with clinically diagnosed meningitis (n = 18) or arthritis (n = 2). They were divided into four categories according to the results of conventional bacteriological analysis. Samples in category I (MenNet05, 06, 07, 09, 10, and 19) were culture positive for *N. meningitidis* (culture-proven meningococcal infections). In category II (MenNet08 and 13), culture was negative but direct smear and/or antigen detection for *N. meningitidis* was positive (possible meningococcal disease). The third category contained samples that were culture negative but showed positive direct smear and/or antigen detection for another bacterium (samples MenNet04 and 12) (Table 1). The fourth category included samples (MenNet01, 02, 03, 11, 14, 15, 16, 17, 18, and 20) that were negative by culture, antigen detection, and direct smear. Conventional bacteriology confirmed the diagnosis in 10 cases (50%), 8 (40%) of which were due to *N. meningitidis* (Table 1).

Molecular diagnosis of *N. meningitidis*. All participating laboratories first carried out a meningococcal screening PCR; four laboratories (L1, L2, L5, and L10) targeted the *ctrA* gene and four laboratories targeted the *crgA* gene (L3, L7, L9, and L11). Two of the latter laboratories (L7 and L9) used additional secondary target genes for the identification of *N. meningitidis* (*crgA* plus 16S rRNA or *crgA* plus IS*1106*). Two laboratories (L4 and L6) used a 16S rRNA gene PCR. L8 amplified *porA* and 16S rRNA genes as a secondary target (Table 2). Genogrouping was subsequently performed with oligonucleotides designed to amplify the *siaD* and *mynB*/*sacC* genes. We first analyzed the congruence of the data among participants (i.e., the consensus results). These consensus results (Table 1) confirmed the diagnosis of *N. meningitidis* in 15 cases (75%). For samples that were positive by culture, latex, or direct smear detection, a good correlation was observed between conventional bacteriology techniques and molecular diagnosis, with the exception of sample MenNet19. The culture method showed that this sample contained an *N. meningitidis* serogroup C strain. Nine of the laboratories failed to detect meningococcal DNA in this sample. One laboratory (L3) identified *N. meningitidis* genogroup C, and another (L2) only detected meningococcal DNA but did not determine the genogroup (Table 1). In eight cases (40%), meningococcal infection was confirmed by PCR alone. Two samples that were positive by conventional bacteriology for other bacterial agents (samples MenNet04 and 12 that were positive for *S. pneumoniae* by latex agglutination and smear positive for gram-positive diplococci, respectively) were negative in the meningococcal PCR assays. The sequences of the VR1 and VR2 regions of *porA* determined by laboratories 6 and 8 suggested that MenNet13 and MenNet14 contained a serogroup A strain, in agreement with the consensus results (Table 1). However, L8 failed to detect serogroup W135 in MenNet06 by using this sequencing approach, which suggested the presence of serogroup B (Table 1).

Performance of nonculture diagnostic methods. The scores of each individual laboratory in relation to the consensus results for each sample were between 55 and 100% (mean, 89.1%) (Table 1). We calculated the sensitivity and specificity of each participating laboratory for the identification of *N. meningitidis* by using the identification consensus results as a reference (Table 1 and Fig. 1). Almost all laboratories obtained good scores when only the detection of *N. meningitidis*

TABLE 3. Oligonucleotides used for genogrouping

Laboratory(ies)	Target gene for genogrouping (genogroup[s])	Oligonucleotide sequences (5′–3′)	Size (bp)
L3, L7, L8,[a] L9, L11	*mynB*/*sacB* (A)	CGCAATAGGTGTATATATTCTTCC CTGAATAGTTTCGTATGCCTTCTT	400
	siaD (B)	GGATCATTTCAGTGTTTTCCACCA GCATGCTGGAGGAATAAGCATTAA	450
	siaD (C)	TCAAATGAGTTTGCGAATAGAAGGT CAATCACGATTTGCCCAATTGAC	250
	siaD (Y and W135)[b]	GGTGAATCTTCCGAGCAGGAAA AAAGCTGCGCGGAAGAATAGTG	342
	siaD (Y)	CTCAAAGCGAAGGCTTTGGTTA CTGAAGCGTTTTCATTATAATTGCTAA	120
	siaD (W135)	CAGAAAGTGAGGGATTTCCATA CACAACCATTTTCATTATAGTTACTGT	120
L4	*mynB*/*sacB* (A)	AAGATGATGCTAGAGGCACT GGATGGTTTTCGAGCGTGT	243
	siaD (B)	ACACCATTACTCTCACCCTCAAC CTTGGATCATTTCAGTGTTTTCCAC	103
	siaD (C)	TTGGACTGACATCGACTTCTATTGTT GGTGTTCTCTTGTTGGGCTGTAT	119
	siaD (Y)	AAGGTGAATCTTCCGAGCAGGA GATATCGTACACCATACCCTCTAGA	110
	siaD (W135)	AAGGTGAATCTTCCGAGCAGGA TTAAAAACACAACCATTTTCATTATAGTTACTGTA	184
L1,[c] L2,[c] L5, L8,[c] L10[c]	*siaD* (B)	TGCATGTCCCCTTTCCTGA AATGGGGTAGCGTTGACTAACAA TGCTTATTCCTCCAGCATGCGCAAA (probe)	170
	siaD (C)	GATAAATTTGATATTTTGCATGTAGCTTTC TGAGATATGCGGTATTTGTCTTGAAT TTGGCTTGTGCTAATCCCGCCTGA (probe)	149
	siaD (Y)	GGTGAATCTTCCGAGCAGGA GGGATATCGTACACCATACCCTCTAG AGCCTTCGCTTTGAGATGTCATGATTAGGATATCTG (probe)	110
	siaD (W135)	GGTGAATCTTCCGAGCAGGA GAATATCATACACCATGCCTTCCATA ATCCCTCACTTTCTGATGTCATGATCAGGATATCTG (probe)	109
L6	*siaD* (B)	CTCTCACCCTCAACCCAATGTC TGTCGGCGGAATAGTAATAATGTT	457
	siaD (C)	GCACATTCAGGCGGGATTAG TCTCTTGTTGGGCTGTATGGTGTA	442
	siaD (Y and W135)[d]	CAAACGGTATCTGATGAAATGCTGGAAG TTAAAGCTGCGCGGAAGAATAGTGAAAT	698

[a] L8 used oligonucleotides for genogroups A, Y, and W135.
[b] Designed by L3 for this study.
[c] L1, L2, L8, and L10 used only oligonucleotides for genogroups B and C (without probes for L8).
[d] The genogroup Y PCR product is cleaved by XbaI into two fragments of 438 and 260 bp.

was considered (Fig. 1A). The mean sensitivity and specificity were 89.7 and 92.7%, respectively. The performance of each of the four genes used (*ctrA*, *crgA*, 16S rRNA, and *porA*) for the identification of *N. meningitidis* was analyzed by comparing the results from laboratories using the same gene target. The mean sensitivity and specificity were similar for all of these targets (Fig. 2). However, the small number of laboratories using each type of target, in particular those that used *porA* (one laboratory) and the 16S rRNA genes (two laboratories), may hinder a reliable comparison.

Each laboratory correctly determined the genogroups of between 30 and 95% (mean, 73%) of strains relative to the consensus (Tables 1 and 3). In several instances, genogrouping by PCR failed and the final diagnosis remained *N. meningitidis*. This was in part because some of the laboratories (L1, L2, and L10) only performed B and C genogrouping, as these are the most frequent serogroups in Europe (Table 1). The results were less consistent for genogrouping than for identification of

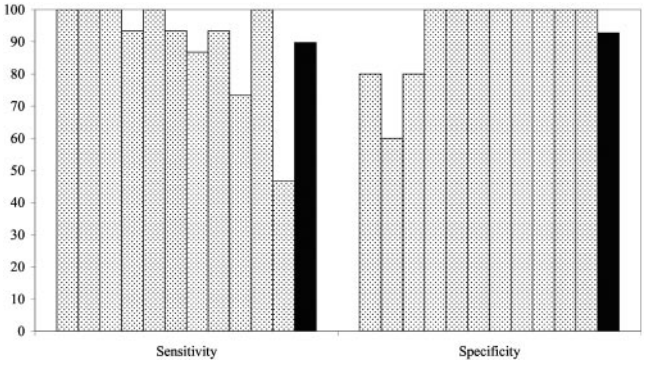

FIG. 1. Sensitivity and specificity of *N. meningitidis* identification methods. Shaded bars represent individual scores for each laboratory, and black bars are the mean values for sensitivity and specificity for all participating laboratories. Individual values were calculated relative to the consensus results for the identification of *N. meningitidis* by using two-way tables as the percentage of samples that achieved positive (sensitivity) and negative (specificity) consensus results in each laboratory.

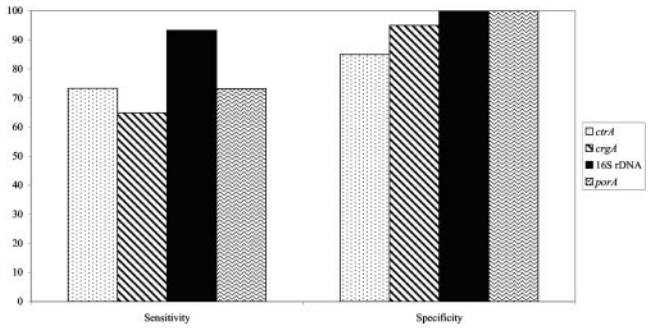

FIG. 2. Sensitivity and specificity of the four genes used to identify *N. meningitidis*: *ctrA* ($n = 4$), *crgA* ($n = 4$), 16S rRNA (two laboratories used only 16S rRNA for identification), and *porA* ($n = 1$). Values were calculated as described for Fig. 1.

N. meningitidis (Table 1 and Fig. 1). The mean sensitivity was 72.7%, indicating that genogrouping was less sensitive than PCR-based identification of *N. meningitidis*.

The genogroup B PCR, which was performed by all of the laboratories, gave the best score (the mean score of correct identification among all laboratories was 85.4%), and two samples (MenNet02 and 03) were correctly identified by all laboratories (Table 1). Lower scores were obtained for the other genogroups, particularly for genogroups A and W135 (mean correct scores were 45 and 50%, respectively). However, only eight laboratories performed A, Y, and W135 genogrouping, and the number of samples belonging to these genogroups was small (Table 1). The participating laboratories obtained good scores for the meningococcus-negative samples (mean score was 87.4%).

DISCUSSION

PCR-based methods are increasingly used to diagnose meningococcal infections and are considered to be rapid and reliable methods for laboratory confirmation and epidemiological surveillance, especially when culture-based methods fail. The standardization of the different PCR-based diagnostic approaches may help to suggest common schemes and algorithms for the management of these life-threatening infections. The European Monitoring Group on Meningococci (a consortium of microbiologists and epidemiologists working in reference laboratories in Europe that ensures the exchange of information on meningococcal infections) and the EU-MenNet provided the ideal opportunity to compare the PCR-based methods used in different laboratories to confirm meningococcal infection. We found that 75% (15 of 20 samples) of our samples were PCR positive for *N. meningitidis*, whereas only 40% (8 of 20 samples) were positive according to conventional bacteriology techniques.

The samples tested in this study were collected, boiled, and centrifuged before being sent to the participating laboratories. It may be better to send frozen samples, although the bacterial DNA in boiled samples seemed to withstand transport at room temperature quite well. It is not essential to purify DNA from CSF unless a PCR-inhibitory effect is observed. Only one sample that was culture positive for *N. meningitidis* (MenNet19) gave a negative consensus PCR result. This may be because the genomic DNA was altered during transport or because a very low concentration of target DNA was present in the sample. However, the percent agreement with each consensus result varied between the tested samples and between the participating laboratories (Table 1). Differences in sample handling and preparation may be, at least in part, responsible for the variable results obtained for some samples. Improvements of the DNA extraction procedure and their impact on the performance of the PCR-based diagnostic methods were not analyzed in this study. A new study for this purpose is currently being designed.

The two-step approach (identification of *N. meningitidis* followed by genogrouping) seems to provide rapid confirmation and serogroup information for the immediate management of meningococcal infections: treatment of the case and prophylactic measures (vaccination and/or chemoprophylaxis) among contacts to prevent secondary cases.

The genes used for nonculture identification of *N. meningitidis* (*ctrA*, *crgA*, *porA*, and 16S rRNA) gave equivalent results, and genogrouping for serogroup B gave the best correlation among the participants.

The performance of genogrouping for the other serogroups varied among the participants, particularly for serogroups A and W135. We recommend the use of the oligonucleotides used by L3, L7, and L4, which correctly predicted serogroup A (Table 1 and Table 3). It should be noted that in many European countries, invasive disease due to serogroup A is rare. Therefore, due to the cost-benefit ratio, many reference laboratories do not stock the reagents required for nonculture detection of serogroup A meningococci.

We found that *porA* sequencing successfully detected serogroup A strains. However, this assay may be less reliable for other serogroups, particularly in an endemic disease situation. Strains of serogroup A tend to express less variable polymorphic serotypes (PorB) and serosubtypes (PorA) than strains of serogroups B, C, Y, and W135 (1, 24). Due to a high rate of recombination, genes are randomly associated on the *N. meningitidis* chromosome, resulting in a low level of linkage disequilibrium between meningococcal genes. Hence, the correlation between serogroup and serosubtype is not perfect (13, 23).

The development of specific and sensitive PCR methods is highly important for the management of meningococcal infections, particularly for urgent diagnosis, large-scale epidemiological surveillance during outbreaks, and when culture methods fail. Moreover, multilocus sequence typing can also be used as a nonculture approach directly on clinical samples (11, 22). However, nonculture assays for the diagnosis of meningococcal disease should not be considered a replacement for culture, as viable bacteria provide crucial information about meningococcal phenotypes, including antibiotic susceptibility patterns, and the pathophysiological behavior of the *N. meningitidis* strain.

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